2ND INTERNATIONAL WORKSHOP ON ACUTE HIV-1 INFECTION

Holiday Inn Select, Bethesda, MD

May 3–4, 2004

Summary Minutes

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Meeting Summary

Monday, May 3, 2004

(8:30 a.m.)

Introduction

Dr. Rick M. Hecht, University of California, San Francisco
Dr. Hecht opened the meeting, pointing out that key events during early HIV infection—e.g., viral load and rate of decline of CD4 cells—determine much of the course of the rest of the disease. Investigators need to address:

- Pathogenesis—the role of innate immune responses, adaptive immune responses, deleterious effects of some immune responses, e.g., T-cell activation.
- Treatment—whether intervention improves immune control and alters disease course, how early treatment should be started, how long treatment should continue, which treatment is best, how treatment should be stopped.
- Prevention—vaccine design (effective immune responses that control viral replication; identifying failures of protective immunity); transmission of drug-resistant HIV .

As one indication, Dr. Hecht used the number of presentations on acute HIV infection at the annual Conference on Retroviruses and Opportunistic Infections from 1997 to 2003. He found a steady increase ($R = 0.99$), indicating a growing scientific interest in early HIV infection. An additional indication is NIH's strong commitment to research in this area for the past 7 years through the AIEDRP.

Goals for this meeting are to determine where we are and where we need to go, especially to overview of current research findings and disease staging techniques, and to identify potential interventions that should be evaluated and key studies that should be done in next 3 to 5 years.

Plenary 1: The Potential Significance of CTL Escape in Acute versus Chronic HIV Infection

Speaker: Dr. Philip Goulder, Partners AIDS Research Center
Moderator: Dr. Rick M. Hecht, University of California, San Francisco

Some 5 amino acid substitutions could occur in one year. It is important to note that extra-epitopic CTL-mediated sequence changes occur outside epitopes, but affect what happens inside. What we know about epitope changes includes the following:

- A change in epitope (e.g., HLA-B27-KK10, generated during acute infection) appears to result in conversion.
- Compensatory changes precede escape.
- Dimerization of capsid protein plays a role.
- Escape appears to predate loss of control, rather than loss of immune control precipitating escape.
- Late escape is associated with effective control of HIV.
- Escape mutations are costly to the virus, so natural selection would favor viruses without escape mutations that may replicate at low levels.
- Simultaneous compensatory mutations reduce the cost of escape.
- Attritional loss of other anti-HIV immune responses reduces cost of escape.
- Effective control of viremia is associated with HLA-B57 and 5801.
- For the patient, escape means loss of reduction of fitness.

Whether escape occurs early or late in infection has implications for vaccine design, as does identification of CTL targets, sites of escape, and acute escape epitopes. Acute escape occurs in 2 or 3 epitopes and identification of these epitopes may be critical to vaccine design. However, the extent of acute escape is still unknown. It seems to be determined by the intensity of CTL efficacy as well as viral fitness. Late escape may precipitate loss of immune control. The goal of vaccine research should not be to address all epitopes, but to reduce escape of acute infection for certain epitopes. To understand correlates of protective immunity we still need to understand the totality of the immune response and more of the details of acute infection.

Discussion

- Theoretically the virus could adapt to a compensation mutation, but, as yet, researchers haven't seen intra-patient reversion for B57 epitope.
- The research emphasis is on one escape, but CTL responds to a variety of epitopes. Escape mutations are generated continuously, but they're not being selected. Replication may occur at a rather low level. We know escape alters selection, but we need to know what else contributes and whether HIV infection could depend on a single epitope or escape of a single epitope.
- Typically within the first month or 2 of infection, the patient recognizes 6 to 20 epitopes before a turning point.
- For TW10, escapees remain at low levels, and in one study about 18 of 20 adults showed no recognition of autologous variants; children recognize more.
- When mothers were sequenced retrospectively, it was found that escape occurred, but that other specificities occur in the same area.

Session 1: Disease Staging

Co-Chairs: *Dr. John Kaldor, National Centre in HIV Epidemiology & Clinical Research, University of New South Wales*
Dr. Brandee Pappalardo, Blood Systems Research Institute, San Francisco

The Screening and Tracing Active Transmission (STAT) Program: Combining Real-time Detection of Acute HIV Infections with Rapid Network Notification in North Carolina

Dr. Christopher Pilcher, University of North Carolina at Chapel Hill, Center for AIDS Research
The STAT program was instituted in North Carolina in November 2002. North Carolina has names reporting, unique identity-based confidential testing, and volunteer partner notification. All publicly funded voluntary counseling and testing (VCT) uses a centralized state laboratory. The North Carolina Health Department collaborated with the UNC CFAR to implement HIV RNA screening of all antibody-negative testing specimens and STARHS testing of antibody-positives to detect incident infection via routine HIV testing. Of 109,250 samples from at-risk patients, 606 were found to HIV infected; 23 (4%) were antibody-negative acute infections detected only by RNA testing and 103 (17%) more were identified as recent by STARHS. 2 HIV uninfected clients had false positive RNA results (overall antibody-NAAT algorithm specificity 0.9999). All acutely infected patients received counseling and referral services. Surveillance activities included quarterly monitoring, geo-mapping by ZIP code (even in rural areas, incidence increased along trucking routes), resistance testing, weekly case review, contact network mapping of likely transmitters. Outbreak investigations were initiated including one that identified a previously undetected increase in incidence among NC youth. NAAT improves performance of public VCT. Standard antibody tests appear insufficient to exclude HIV infection in many populations. Very large-scale identification of acute HIV is possible using pooling and RNA screening for VCT, even in low-prevalence areas like North Carolina. But to do this effectively, the public health community and the clinical research community must be directly linked.

Discussion

- Blood has been collected, but so far no correlations have been made to document transmission between individuals.
- The delay between testing and notification is about 10 days; about two-thirds received treatment.
- Lab testing, and cooling equipment result in a cost of about $2 per test, double what the state lab spends for HIV screening.
- Reporting new cases of HIV is mandatory. In total, 800 new reports per year come from the state lab and about 600 from private labs.

Specimen Pooling/RNA Testing Algorithms for HIV Voluntary Counseling and Testing (VCT)

Daniel Westreich, University of North Carolina at Chapel Hill, Center for AIDS Research
Surveillance for acute HIV with nucleic acid amplification testing is expensive; to reduce costs and increase specificity and positive predictive value in its routine HIV VCT, North Carolina has added routine pooled screening for HIV viral RNA for specimens found antibody-negative with ELISA testing. Using a simple pooling algorithm, they calculate an approximate sensitivity loss of 10% compared to individual testing (from 75% to 68%, overall). Simple mathematical models show that efficiency (number of PCR test kits consumed) is highly dependent on prevalence; savings are about 98% of test kits in North Carolina, and about 80% in an African STD clinic. The positive predictive value gained with this assay over individual testing is immense in both the North Carolina and the African STD clinic. The exponential growth of viremia during early acute infection results in little loss of sensitivity, but substantial cost savings result from pooling strategies, especially in low-prevalence settings. Smaller pools are required to efficiently screen higher-prevalence populations (e.g., domestic emergency departments or African STD clinics). And the pools can be easily created with a hand-pipette.

Discussion
- As prevalence rises, efficiency falls. Blood screening entails different issues than diagnostic screening.
- West Nile diagnosis was treated similarly this year in the United States.
- Contamination is a serious concern in these estimates; additionally these estimates do not account for imperfect screening tools.

A Simplified Method for Detection of Incident HIV Infection

Dr. Tim T. Ramacciotti, National Center in HIV Epidemiology and Clinical Research, University of New South Wales
A method using Western blot banding patterns to classify newly diagnosed HIV infections as incident or established was compared with detuned EIA scores for the same patients. The bands in Western blots were measured for 4 groups of patients for gp160, gp120, p68, p55, p53, gp41, p34, p24, and p18; scored as negative, trace positive, or 1+, 2+, or 3+ positive; and summed (intensity score). Using the Western blot patterns and intensity score may offer a fast, efficient, and inexpensive method to monitor incident HIV infection.

Discussion
- There is some flexibility around lower levels of infection.
- The method should be applied to a larger cohort, and is being test in the Phaedra cohort.
- p31 appears to be very important.
- Intensity is important.
- Western blot technique entails not only operator variability, but also manufacturer variability.

Session 2: Immunology I

Co-Chairs: *Dr. Marcus Altfeld, Massachusetts General Hospital*
Dr. Philip Goulder, Partners AIDS Research Center

HIV-1 Induces Persistent Changes in Peripheral γδ T Cells as Early as the Primary Infection Period

Dr. Michael A. Poles, Aaron Diamond AIDS Research Center and New York University School of Medicine

γδ T cells are components of innate immunity and play a major role in a variety of infectious diseases. They kill infected cells and the pathogens themselves and may produce Th1 or Th2 cytokines to initiate and regulate adaptive response. Additionally, they promote antibody production by B cells. Two of the most common subsets are Vδ1 γδ T cells and Vδ2 γδ T cells. We studied 43 patients (39 men and 4 women) who were diagnosed with acute HIV-1 infection for a duration of 28 days, 14 days prior to development of symptoms of disease. We noted that in mucosa, but not in the peripheral blood, the number of γδ T cells increased among those infected with HIV-1. HIV-1 infection was also found to be associated with significant expansion of Vδ1 and contraction of Vδ2 γ δ T cell populations in the mucosa and in the peripheral blood. These changes are observed during acute HIV-1 infection and persisted throughout the chronic phase of disease, without apparent reversion despite prolonged treatment with HAART. Future research should be directed to determining mechanistic sources of these changes, and to examining the functional consequences of phenotypic changes in mucosal and blood γδ T cells.

Discussion

- It may be possible to use monoclonal antibodies to determine the γδ T cell role.
- All HAART-treated patients maintained viral suppression, but reversion of γδ T cell didn't change.
- These cells can be infected by HIV. A significant subset expresses CD4, CCR4, and CCR5, but the role of HIV-1 infection of γ δ T cells, in the depletion of Vδ2 cells is yet to be determined.
- Sexual behavior could play a role in the observed changes in γ δ T cell phenotype, but seronegative patients were recruited from Bellevue Hospital, and most were infected by intravenous drug use.
- These cells express increased mucosal homing-receptors, such as the β7 inetgrin and CCR9.
- MHC-like molecules that are expressed on epithelial cells in response to stressors, such as infection may play a role in the expansion of the V δ 1 γ δ T cell population.

NKT Cells Are Preserved in Patients Beginning HAART during Acute Infection

Dr. Sandhya Vasan, Aaron Diamond AIDS Research Center

Natural killer T cells (NKT cells), which share features of NK cells and of T cells, are another arm of innate immunity. They are similar to T cells, but posess a semi-invariant

T cell receptor. In humans, the alpha chain is always V α 24, and the beta chain is predominantly, but not always, V beta 11. NKT cells are thymus dependent for maturation but, like NK cells, they are not restricted by MHC. Their actions include suppression of hepatic metastasis of primary melanomas, suppression of autoimmune diseases (e.g., diabetes, SLE, MS), contribution to the granulomatous reaction of *M. tuberculosis,* and protection against murine malaria. However, HIV can infect NKT cells, and levels of both NKT cells and the $CD4^+$ NKT cell subset decline rapidly within the first 2 or 3 months after infection. Initiating HAART prevents further depletion of both, although it doesn't restore the lost cells, and NKT cell function may be preserved by early initiation of HAART.

Discussion

- Previous studies show no direct correlation between NKT and viral load or prognosis. NKT cells are known to kill other infectious agents, so they could decrease co-infections, and they may be necessary to kill HIV.
- The absolute number of NKT cells shows the same pattern.

Dendritic Cells in Early HIV-1 Infection

Dr. Michael S. Killian, University of California, San Francisco

Plasmacytoid dendritic cells (PDC) are important in terms of adaptive and immune response. They are the main producers of interferon-α, a potent inhibitor of viral infections, but the cells are susceptible to HIV infection and PDC counts are highly predictive of opportunistic infection. To examine the impact of HIV on PDC, dendritic cells were characterized in peripheral blood by cytofluorometry and subjects were followed for 48 weeks. There was no significant change in viral load, but a significant decline in CD4 cells (about 1% every 3 weeks). The number of cells inversely correlated with viral load, but was not substantially correlated with $CD4^+$ cell count. Interferon-α was not correlated with PDC levels: interferon-α did not change over time, although PDC decreased significantly in primary HIV, as did CD4 cells. During acute HIV-1 infection individuals have mean levels of PDC significantly lower than seronegative subjects.

Discussion

- IL-3 receptor expression has not yet been examined.
- In long-term nonprogressors, the exact timing of cell decline has not been shown.

Stereotypic $CD8^+$ T-cell Responses during HIV-1 Sequence Evolution

Dr. Todd M. Allen, Partners AIDS Research Center

CD8 T-cell responses drive viral evolution in the host and are related to several mutations (*gag, pol, vif, vpr, vpu, env,* and *nef*). More than 50% of mutations outside *env* are associated with CD8 T-cell responses. Some 12% may represent mutations reverting to a consensus sequence. However, few CD epitopes have been identified as yet. In this study, CD-8 T-cell responses were monitored to elucidate the relationship between CD8 escape mutations in the host and sequence variability. Using autologous peptides

against regions exhibiting sequence variation, 3 novel CD8 responses were identified. In the kinetics of CD8 escape and reversion, TW10 escapes early and reverts early. CD8 responses are associated with mutations developing over the course of infection. Escaping residues reflect the most polymorphic sites in epitopes in circulating HIV-1 strains. Viral escape appears to be stereotypical with escaping residues. Biochemical structure of viral proteins may dictate where and when escape occurs. Additional CD8 T-cell responses may be detected against some of the remaining sites of sequence variation.

Discussion

- Mutations are relatively biochemically conserved, and in some patients are not the exact same residue.
- Other issues dictate what residue finally gets changed.
- Molecules are classified in groups called super-motifs, but patterns of immunodominance, e.g., for B57, are not clear.
- CD4 data need to be analyzed and the protein crystal overlay done.
- Proteins with more mutations are more variable.

Viral Infection Persistence, but Not Level of Viremia, Leads to Ablation of HIV-specific CD4 and CD8 T Cells

Dr. Rafick-Pierre Sékaly, University of Montreal

Assays to analyze HIV-specific CD4 and CD8 immune responses and their persistence elicited strong CD4 and CD8 proliferative responses against individual peptides. Env responded to both CD4 and CD8, and there was gated response on $CD4^+$ $CD69^+$. Potent $CD4^+$ responses can identify HIV infection. In long-term HAART-treated patients, after less than 1 month's exposure, CD4-specific responses are low but peak at 30 to 90 days. Untreated patients show diverse HIV-1-specific CD4 and CD8 T cells 30 to 90 days after infection. There is a negative correlation between breadth and magnitude of viral load with CD4 cells, and a time-dependent decrease in the number of HIV-specific epitopes. In patients treated before initiation of immune response, HIV-1-specific CD4 cells are not preserved. In patients treated within 30 to 90 days, preservation is better. HIV-1-specific proliferating $CD4^+$ and $CD8^+$ T cells are present in patients with primary infection, with or without HAART. These cells proliferate despite a high viral load but disappear as the virus persists. The conclusion is that viral persistence, and not viral load, determines the decrease in the number of epitopes.

Discussion

- Escape mechanisms will be studied. The investigators are currently testing selective pressure of CD8 to modify CD4.
- Timing of when you look for responses is very important and determines what you see, regardless of the assay used. There's a predominance of Gag responses and γ-interferon cells.
- In the proliferation of CD4 and CD8, many responses appear to be the same, but these studies can't yet prove that.

- Data support timing of treatment (too early doesn't result in prolonged response). Seroconversion may not yet have occurred at 30 to 90 days when treatment begins.
- They plan to look at this in patients who stop therapy.

Host Factors and HIV-specific Immune Responses

Dr. Souheil-Antoine Younes, University of Montreal

The quality of CD4 response differs when patients are treated early than when they are treated a year after infection. Moreover, early HAART-treated patients show a much broader response than late-treated patients. This study examined the proliferative capacity of HIV-specific $CD4^+$ T cells in aviremic and viremic patients. CD4 cells reside in lymph node until infection, and there is no positive correlation between the number of TREC and the number of CD4 cells. $CD4^+$ T-cell responses are peptide-specific. Central memory CD4 cells express CCR7, IL-2, and IFN-γ, a broad response that competes with CD8. Many cells produce IL-2 but not IFN-γ, and the IL-2-producing cells maintain the same frequency over time. Central memory cells are detected only in aviremic early-treated patients after prolonged HAART therapy. Chronic activation and the lack of central memory HIV-specific CD4 cells may impede immune control of HIV infection. Data from this study support the hypothesis that HIV-specific $CD4^+$ T-cell depletion is mediated by chronic immune activation that prevents the establishment of IL-2-producing, long-lived memory cells. Early HAART initiation favors the maintenance of central memory $CD4^+$ T cells.

Discussion

- Adult patients treated late don't mount memory response.
- The problem is the frequency of the restriction element cells, not the classification.

Novel and Promiscuous $CD8^+$ T-cell Epitopes in Conserved Regions of Subtype C Recognized during Early HIV-1 Infection

Dr. Agatha Masemola, National Institute for Communicable Diseases

To investigate the pathogenesis of HIV in southern Africa, this group analyzed HIV-1 infected individuals for CD8+ T cell responses using overlapping peptides spanning the whole HIV genome to identify epitopic regions. Of 46 patients, 44 (96%) responded to one or more HIV-1-derived peptide. There is a positive correlation between plasma viremia and the total magnitude of HIV responses, which is driven by recognition of Nef. Preferential targeting of Gag is important for control of HIV replication. Optimal epitopes occur in in conserved regions of Gag, overlapping or embedded within subtype B described epitopes. Although conserved regions in Gag were targeted in both subtype B- and C-infected individuals, the HLA background of the individuals dictates the epitopes that are presented, some of which may be important in control of viremia and establishing viral set-point. Data from this study highlight the importance of identifying CTL epitopes in geographic areas at the epicenter of the HIV epidemic for the rational design of a CTL-based vaccine.

Plenary 2: The Early Evolutionary Dynamics of HIV Infection

Speaker: *Dr. James Mullins, University of Washington School of Medicine*
Moderator: *Dr. Bruce Walker, Harvard Medical School; Massachusetts General Hospital–East*

Over the first 3 months of infection of men with Clade B virus, the virus population achieves homogeneity at the env locus, in a process that likely involves transfer of multiple infecting viruses and selection (acting particularly on Env protein) for the fittest variants in the new host. Over the first 2 to 3 years, there is a gradual drift of the viral sequence toward a consensus-like, most recent common ancestor. Although not responsible for most of this drift, over the same period, CTL-driven mutations occur and CTL become a/the dominant selective force shaping the virus population. There is evidence that diversifying selection sites (33 were found in this study of one patient) change gradually over time, whereas directional selection (sudden changes) was found at 31 sites. Forty-two CTL epitopes were found, as were 18 escape mutations, and 15 escape-selection matches. Also observed was a single reversal of a CTL escape mutation. It is possible that change in such epitopes has a significant fitness cost. It is also possible that no advantage is gained within the first 3 years of infection. Therefore additional mutations may affect utility and presentation of the epitope. Epitope forms have a higher database frequency than mutant forms, and epitopes are likely to occur at sites of high entropy.

Discussion

- The mechanisms are unclear for reversion to the most common ancestor.
- Data come from the proteins Env, Nef, p24, and Tat, but effects are most pronounced in Env.

Session 3: Immunology II

Co-Chairs: *Dr. Jay Levy, University of California, San Francisco*
Dr. Cara Wilson, University of Colorado, Health Sciences Center

Sexual Transmission of HIV-1—Antigenic Characteristics Either Side of the Transmission Boundary

Dr. Rodney E. Phillips, University of Oxford

Infection was thought to be established by a small founder set of clones and that genetic diversity occurs at transmission. Transmitter-couple studies show that transmission is not by phylogenetic but by contact type. The virus in the donor and in the recipient is similarly divergent, whereas in an unrelated seroconverter, diversity occurs at different sites with different amino acids. It can be concluded that the HLA-I–relevant antigenic characterizations are transmitted sexually; that antigenic diversity is transferred sexually; that HLA-sharing, preformed escape sequences are further propagated; and that overlapping sites of distinct restriction may escape through a single polymorphism. Close inspection of HIV obtained from donor and recipient homosexual pairs at the

time of successful viral transmission reveals that the extent of antigenic variation transmitted is substantial; it provides a formidable, probably insuperable challenge to the type of vaccine-induced immunity evoked by approaches that rely on simple, consensus antigens.

Discussion

- Over time, as the HIV epidemic has matured, HIV seems not to have become more pathogenic. There seems to be a strong tendency to revert back to something like the consensus, but with no sharing or overlap there would seem to be no advantage.
- HLA disparity might weaken the virus, which would fit with the escape model of common alleles restricting common genes.

Neutralization Profiles by Monoclonal Antibodies 2F5, 2G12, and 4E10 of Plasma-derived HIV-1 from 91 Newly Infected Individuals

Dr. Saurabh Mehandru, Aaron Diamond AIDS Research Center

Monoclonal antibodies have been developed to epitopes of gp41, 2F5, 4E10, gp120, and 2G12. Neutralization profiles of plasma-derived pre-treatment HIV-1 were assessed for 91 newly infected patients (90 MSM and 1 woman). All newly transmitted HIV-1 isolates were susceptible to neutralization by 4E10 and the majority was also susceptible to neutralization by 2F5; and 37% was susceptible to neutralization by 2G12 but these isolates were less susceptible to 2G12 neutralization than to NL4-3 and JRCSF. Only 37% were susceptible to neutralization by all 3 monoclonal antibodies; but 86% were susceptible to at least 2. Clinical trials are warranted for high-affinity 4E10 antibodies as a preventive vaccine.

Discussion

- None of the other monoclones work as well as E410; 4E10 is unique.

Immune Control and Escape: Characterization of Neutralizing Antibody Responses to HIV-1

Dr. George M. Shaw, University of Alabama at Birmingham

The antibody response to HIV-1 infection is typically vigorous and sustained but its effectiveness in virus containment *in vivo* is uncertain. The researchers have shown in acutely infected individuals the rapid development of HIV-1 strain-specific neutralizing antibodies (Nab), and the equally rapid emergence of virus escape mutations. Such strain-specific antibody responses are common, and they clearly drive virus selection *in vivo.* More broadly reactive Nabs develop over longer periods. Analysis of HIV-1 specific monoclonal antibodies has revealed variable loop, CD4 binding site, chemokine co-receptor binding site, surface glycan, and gp41 domains as neutralization targets, but the prevalence, titers, and breadth of polyclonal responses to these epitopes in humans are generally unknown, largely due to technical difficulty in identifying epitope-specific neutralizing antibody responses within a larger context of polyclonal neutralizing and non-neutralizing antibody reactivities. In the present study, the researchers have taken advantage of the wide evolutionary distance that exists between HIV-1 and HIV-2 lineages to probe for conserved neutralization

epitopes. The envelope glycoproteins of HIV-1 and HIV-2 are only about 40% homologous in amino acid sequence. As a consequence, they exhibit weak antigenic cross-reactivity, and sera from HIV-1 infected individuals cross-neutralize HIV-2 poorly if at all. Nonetheless, HIV-1 and HIV-2 each require chemokine co-receptor binding for cell entry, with primary non-T cell line adapted viruses of both types generally utilizing CCR5. The researchers hypothesized that conserved requirements for co-receptor binding might be reflected in conserved antigenicity at this envelope surface. Here, they show that the chemokine co-receptor binding site of HIV-1 from clades A, B, C, D, F, G and H, and circulating recombinant forms CRF01, CRF02 and CRF11, elicits high titers of CD4-induced (CD4i) antibody during natural human infection and that these antibodies bind and neutralize viruses as divergent as HIV-2. CD4i monoclonal antibodies elicited by HIV-1 infection, including 17b, 21C, 19e, 31H, E51, ED49, ED47 and X5, bind and neutralize HIV-2 pretreated with soluble CD4, and polyclonal antibodies from HIV-1 infected humans compete specifically with such monoclonal antibodies for HIV-2 binding. *In vivo,* variants of HIV-1 with spontaneously exposed co-receptor binding surfaces were detected in human plasma; these viruses were neutralized by CD4i antibodies. The researchers find that despite remarkable evolutionary diversity among primate lentiviruses, functional constraints on receptor binding create unexpected opportunities for broad humoral immune recognition, which in turn serves to constrain the viral quasispecies. Future studies will be aimed at determining what role such broadly cross-reactive Nabs have in virus containment in acute and early infection as well as later in disease. In turn, they may contribute to the development of broadly active vaccine immunogens.

Discussion

- Looking at human vaccinées shows loop antigens in addition to neutralizing antibody response to a hidden epitope—it's very immunogenic.
- All 4 clades of individuals elicited an antibody response that will (it is assumed) neutralize HIV-2.

The Incidence of HIV Superinfection following Primary Infection

Dr. Davey Smith, University of California, San Diego

Superinfection is defined as infection first by one strain and then later infection by another strain. Using 4 independent lines of molecular investigation they discerned HIV-1 clade B superinfection, although co-infection can never be ruled out. Superinfection occurred 5 to 13 months after the estimated date of initial infection; all 3 were male and had homosexual exposure as their only HIV risk factor. There was no epidemiologic link with the superinfecting partner. Superinfection negatively affected each individual's clinical course. Initial CTL responses resulted in effective control of the MDR strain, but subsequent exposure to a superinfecting strain led to failure of immune containment due to nonrecognition by strain-specific CTL. The failure of a vigorous antiviral response to prevent superinfection by another strain of the same subtype is a major concern for effective vaccine development, and at the same time, the true rate of superinfection may be underestimated.

Discussion

- If the level of resolution is low, primary specific PCR might be considered.
- We need to talk about terminology (perhaps form a committee?) to evaluate the meaning of superinfection versus dual infection versus co-infection. And, regardless of terms, we need to know whether the condition is clinically relevant.
- A problem is that you don't really know when infection occurred.
- The most rapid of rapid progressors were dually infected persons, although it's not possible to tell whether it was a host or virus effect.
- After primary infection you can still be infected with another strain.

HIV-1 Dual Infection in a Female Sex Worker Cohort in South Africa

Dr. Carolyn Williamson, University of Cape Town

The epidemic in southern Africa is homogenous with more than 95% infected with subtype C. Increased disease progression may be associated with dual infection (infection with 2 phylogenetically distinct HIV strains) or multiple diverse variants. Of 31 South African female sex workers identified as having a dual infection, 25 were monitored via longitudinal plasma viral load measurements for 2 years. Although there was no evidence of superinfection, the viral populations fluctuated. Dual infection was associated with viral load and increased viral set-point. The risk of dual infection was highest close to time of infection. The mechanism may be that transmission of multiple highly diverse variants enables rapid adaptation and immune escape resulting in increased disease progression, or that people who are predisposed to be rapid progressors cannot control viral diversity, enabling establishment of dual infection.

Discussion

- The assay used is great for populations, but not for individuals; you still see bands with chronically infected individuals.

(Whereupon the meeting was adjourned at 4:15 p.m . until May 4, 2004.)

Tuesday, May 4, 2004 (8:30 a.m.)

Plenary 3: Acute Infection in High Prevalence Settings: Opportunities and Constraints

Speaker: *Dr. Salim S. Abdool Karim, Centre for the AIDS Programme of Research in South Africa (CAPRISA), University of KwaZulu-Natal*
Moderator: *Dr. Martin Markowitz, Aaron Diamond AIDS Research Center*

In southern Africa, the HIV epidemic began in the late 1980s to early 1990s and apparently reached a plateau at its peak of ~23% in 2001. However, high death rates camouflage the true incidence rates, resulting in apparently static HIV prevalence rates which create the impression of stability. The epidemic has seen explosive growth in young women; e.g., the incidence rates in pregnant rural women is about 10% per annum. The prevalence rate in men peaks in their late 20s and early 30s, but in women it peaks in their early 20s and decreases with age as they die. In 2001, the prevalence in the 20- to 24-year-old age group of HIV infection was 50.8%. Nevertheless, this bleak situation offers research opportunities in assessing the effect of interventions, clade differences, differences in routes of transmission, as well as the effects of co-infections (e.g., STD), HLA differences, viral diversity (for vaccine development), drug resistance monitoring, pathogenesis and set-point, immune responses, and dual infections. In addition, they can enroll large numbers of patients from pre-funded sources (e.g., HPTN 035), with various subtypes (subtypes A, B, C, and D). Constraints in such settings are: laboratory infrastructure (e.g., power failures), assay validation and shipping of specimens; follow-up of mobile participants (sex workers, truck drivers) in the absence of addresses (they use GPS technology to plot households); ethical issues, such as recruiting adolescents; community buy-in; and treatment issues. There is a pressing need for treatment, but only now are there a range of options to make treatment available, e.g., price reductions from pharmaceutical companies, and generic drugs. To identify acute infection in South Africa, these investigators have used the "detuned" assay in prevalence studies in high-risk populations, antibody detection, symptom identification in STD patients (this was not very successful and was not pursued), and in the CIPRA cohort, RNA-PCR pooled, which looks at viral set-point and clinical progression. Pelvic exams allow them to study mucosal and innate immunity, etc. Their target for 2005 is to have enrolled 152 seroconvertors.

Discussion

- As for identifying HIV infection from symptoms, it is true that patients presenting with STD might have a high probability of also having HIV, but they have so many nonspecific complaints (fever, etc.) that these investigators found no correlation. It might work better in a population without so many other complaints.
- The incidence rate of infection in pregnant women has leveled off and remains close to 10% per annum. With a background of prevalence at 40% and continued

incidence of 10%, this 10% rate can only be maintained by a continuing mortality rate that offset the incidence rate.
- They have had mother-to-child prevention programs, but the problem is a fundamental structural one; namely that Apartheid resulted in conjugal instability with men away from home for long periods, so protecting a woman with condoms (education) is not a practical solution when she wants to become pregnant and seldom sees her husband.
- The relatively low viral load in sex workers can't be accounted for. In this group, some women have not become infected despite much sexual activity. The anomaly shows that you can't study only sex workers.
- Microbicide had no effect on viral load.
- In the 1992 prevention trial, they had nothing to offer women who became infected, but now they have several options to offer treatment, including ART. They have prioritized access to treatment.

Session 4: Virology, Epidemiology, and Research in Resource-poor Settings

Co-Chairs: *Dr. Jean-Pierre Routy, McGill University*
Dr. Roberto Badaró, Hospital Universitãrio Professor Edgard Santos, Federal University of Bahia

HIV-1 V1/V2 and V3 env Diversity during Primary Infection Suggests a Role for Multiply Infected Cells in Transmission

Dr. Kimberly Ritola, University of North Carolina at Chapel Hill Center for AIDS Research
In 109 subjects in this study, sex was the mode of transmission, and homosexual contact was most common. Viral complexity at transmission was assessed by heteroduplex tracking assays of the V1/V2 and V3 regions of *env*. Overall, 50% of subjects harbored multiple viral variants during primary infection. No correlation was seen with the sex of the patient, but with the mode of transmission. All 8 heterosexually contacted men harbored a single variant, while 57% of the 7 women and 55% of the homosexual men were infected with multiple variants. The V3 region was significantly less diverse with only 18% displaying multiple variants, but transmission of X4-like variants was seen in 3% of them. A dual infection frequency of 7% was detected. These findings suggest that the type of mucosal surface exposed (vaginal/rectal versus penile) during infection influences the number of variants transmitted. The high proportion of multiple variants is not consistent with low probability transmission event involving cell-free virus. Transmission may occur via a multiply infected cell.

Discussion
- Some individuals have a better antibody response than others, which could account for the timing of diversification. The *env* region in monkeys showed a similar difference in mode of transmission. These researchers haven't looked for species selection; distance analysis indicates a single donor.

- It is difficult to know when infection occurred, but you can be more sure with people who hadn't seroconverted yet. We may be seeing a difference in recruitment time.

Risk Reduction Behavior of MSM with Recent HIV Infection

Lydia Drumright, MPH, PhDc, University of California, San Diego

HIV incidence among men who have sex with men (MSM) is increasing worldwide. Understanding behavioral change after diagnosis is an important component of prevention. To determine patterns and duration of behavioral change, these researchers studied 84 MSM by intensive behavioral interviewing regarding the 12-month period prior to the baseline interview, using a computer-assisted survey instrument. At a 15-week follow-up interview, risk behaviors were reduced (less concurrency, fewer partners [an average of 8.6 declined to 5.3], selective positioning for anal intercourse by partner HIV status, and high levels of disclosure). The range of partnership types at baseline was still seen at follow-up, although 24% of the primary partnerships had terminated. There was significantly less unprotected anal intercourse with an unknown partner, but significantly more with the main partner. Disclosure increased significantly, especially to a friend and a main partner. However, unprotected anal intercourse between discordant partners still occurred after disclosure, indicating that HIV-negative partners may make a conscious decision to engage in risky sexual activity.

Discussion

- There's a tension between the individual good and the public health good, and there's a tremendous probability of transmission, but current intervention strategies seem not to be working.
- Recreational drugs are a risk factor; for some 24% of participants this was an issue.

Recruitment Strategy to Optimize the Identification of Acute HIV-infected Patients in Bahia, Brazil

Dr. Eduardo M. Netto, Federal University of Bahia, Brazil

The Fever Project entailed active surveillance of HIV infection in all patients who had an acute, nonspecific febrile illness. From these patients, blood was drawn (after informed consent), pooled (10 samples per pool), and tested for HIV. If the pool was positive, the 10 individuals were tested. Arthralgia was the only symptom significantly associated with HIV infection. Active case detection in nonspecific febrile illness is a very effective strategy to identify early and acute HIV infection, especially among people who engage in high-risk activities.

Discussion

- These researchers defined "recent" as 1 to 12 months; and they used a detuned assay.

- Clade infection was mixed between B and C, but the data have not yet been analyzed.

Successful Identification and Recruitment of Acutely HIV-infected Subjects in a Malawi STD Clinic

Dr. Susan Fiscus, University of North Carolina at Chapel Hill Center for AIDS Research
In STD clinics in Lilongwe these investigators are conducting a diagnostic clinical trial for acute HIV infection to compare p24 antigen screening with HIV RNA testing using a pooling strategy for real-time testing. The standard p24 antigen assay was not very predictive, but the HDp24 antigen assay may be. Almost half of patients with discordant rapid test results had acute infection. Semen HIV dynamics confirm the brief but extreme infectiousness associated with acute HIV infection. The public health benefits of making the correct diagnosis at this time are profound. Real-time pooling and RNA testing can identify HIV infection in resource-poor countries. Using pooling and RNA PCR on-site in Lilongwe, researchers accurately identified the approximately 1 in 50 clients in the STD clinic that is antibody negative but acutely HIV infected. However, using p24 antigen testing they detected only 58% of antibody negative, but viremic acute HIV infections.

Discussion
- Discordant results are not related to the type of test used.

Treatment received for STD could relate to the decreasing viral load.

Acute Pediatric HIV Infection and Early HAART/Structured Treatment Interruption: Set-up of a Clinical Research Study in Resource-limited KwaZulu-Natal, South Africa

Dr. Krista Dong, St. Mary's Hospital, Durban
The Pediatric Early HAART/STI Study (PEHSS) was initiated to test the efficacy of early HAART with structured treatment interruption (STI) in acute pediatric infection (infants infected through mother-to-child transmission). A study of this sort requires a high-risk group with high prevalence, a method of identification, and follow-up and adherence near 100%. In the United States at its peak some 700 babies were infected; in KwaZulu-Natal, about 200 babies are infected daily. Because no treatment is yet available, 50% die within 2 years. In 2002, 35% of pregnant women were HIV^+, and in 2003, 51% were. The challenges to clinical research are: tracing (no street names), communication (no phones), emergency transportation (no ambulance service because the crime rate is so high), transportation (vast area), trust (80% use traditional healers), mistrust (at a high government level, ARV are said to be poison), funerals every weekend, and stigma and consequent non-disclosure (women taking nevirapine may not disclose their status at delivery). The main concerns of the surveyed community were unemployment, poverty and food security, risk of violence, and (fourth) HIV infection. To address the HIV infection, we must address the first 3 concerns. They designed a 4-week training course on HIV/AIDS to be given at an antenatal clinic. To date, 229 women have been recruited; 113 babies are eligible for the study. Most (97%)

of participating mothers successfully completed the training course. Adherence to the HIVNET 01 protocol in labor and following birth resulted in a 2.7% intra-partum mother-to-child transmission rate. Traditional medications complicate the issues (e.g., mothers or infants who do well on anti-HIV drugs but die because of additional traditional remedies), but the study shows that frequent follow-up and adherence to HAART can be achieved.

Discussion

- NNRTI resistance and reduced efficacy has been addressed in mothers, but not in infants. There have been no changes in protocols despite newer data. In this study, they stop nevirapine when viral load is undetectable. When babies off nevirapine reach a viral load of 5000 or less, therapy is started again.
- Salvage regimen in adults show that structured treatment interruption doesn't offer improved results. But there are secondary endpoints with infants. The rationale to using structured treatment interruption in infants is to preserve and boost the immature immune system.
- Children respond very differently than adults; this is the first study of HAART in infants. Structured treatment interruption is needed to get the HIV response. They expose the infants to the virus at a time when they can generate T-cell responses. The purpose is to see if it's feasible to do such a study; if so, they can then develop a larger study.

Session 5: Treatment and Resistance

Co-Chairs: Dr. Susan Little, University of California, San Diego
Dr. Rodney E. Phillips, University of Oxford

The Transmissibility of Drug-resistant HIV-1 in Partner Pairs

Dr. Rick M. Hecht, University of California, San Francisco

Drug resistance can be transmitted, but relative transmissibility compared to wild type HIV is unknown. This study investigated selective pressure against transmission of drug-resistance mutations in partner pairs. They tested the source partner and recipient partner, analyzing *pol, gag,* and *env* sequences and comparing frequency of resistance to different drug classes. In 47 transmitting partner pairs, 28% were drug resistant, of whom 13% were PI resistant, 19% NRTI resistant, and 15% NNRTI resistant. In 36 non-transmitting partner pairs, 17% were drug resistant, of whom 17% were PI resistant, 6% NRTI resistant, and 8% NNRTI resistant. Drug resistance to PI, NRTI, and NNRTI mutations were most often transmitted. In this study, no evidence was found of selective pressure against transmission of drug-resistant HIV except for a lower frequency of transmission of the L90M protease mutation. Nearly all resistance mutations present in the source partner were found in the spread partner. These results suggest that there are not substantial barriers to transmission of drug-resistant mutations in any ART drug class.

Discussion

- PI resistance was modest, and may not have been tested enough yet.
- Most people were resistant to only one drug (there were no triple class resistors and only a few dual class). These investigators started accumulating partner pairs in 1998, but recruited most of them in the last 3 years.
- Findings were the same in the Montreal study; PI resistance in 184 patients may be less transmissible.
- A paper on the persistence of drug resistance mutations is in press; many drug resistors were stable over 1 to 2 years, but the PI mutations seem less stable.
- Not directly addressed were exposure behavior and compliance, but a paper on the association between less adherence and risk taking is in preparation.

Long-term Control of HIV-1 RNA Replication following a Unique Supervised Treatment Interruption and Mycophenolate Mofetil Therapy in Patients Treated with HAART since Primary HIV-1 Infection

Dr. Chiara Tassan Din, HSR Hospital, San Raffaele Scientific Institute, Milan

Mycophenolate mofetil (MMF) is a licensed drug used in solid organ transplant. It inhibits proliferation of activated T cells even in the presence of IL-2, and the objective of this study was to assess the effect of MMF on viral load and $CD4^+$ T cells. Treatment consisted of an induction phase (500 mg MMF twice a day) and a maintenance phase (500 mg daily). HAART was begun again when plasma viral load exceeded 1000 copies/mL. HIV RNA rebounds during 24 weeks of MMF therapy. Viral rebound occurs in primary HIV-infected patients after a single structured treatment interruption. Unique structured treatment interruption and 24-week MMF in combination are associated with long-term control of virus replication. MMF in the context of structured treatment interruption apparently does not compromise the efficacy of new HAART and there are no major side effects.

Discussion

- In a Swiss study, structured treatment interruption appeared to do well, but in those patients, pre-treatment viral load was very low, i.e. the patients had not achieved set-point because treatment was started so early in infection. A control group is needed to see whether that is happening in this population.

HIV Immune and Virologic Responses following the Administration of IL-2, either Alone or Combined, to ALVAC-HIV 1433 and HIV Lipopeptides (LIPO-6T) in Patients Treated Early with HAART during Primary Infection: the ANRS 095 Randomized Study

Dr. Cecile Goujard, Bicêtre Hospital, Le Kremlin Bicêtre, France

HIV-specific immune responses are preserved in patients treated early during primary infection. This research evaluated whether the addition to HAART of IL-2 alone or combined with immunization might enhance HIV immune responses and improve viral control after HAART discontinuation. They found no significant differences in virologic

success among 3 groups (N = 43). Immunologic tests used were the lymphocyte proliferation assay and γ-INF ELISpot assay. IL-2 alone or combined with a vaccine was well tolerated but had no deleterious effect on viral load over time, and no effect on CD4 or CD8. Sustained anti-p24 was associated with virologic success. In sum, immune interventions were not statistically significant, but improvement of some virologic parameters, mainly in patients with sustained LPR to HIV antigens, warrants further investigation.

Discussion

- Higher proliferation is associated with success, not failure, but its appearance could be an artifact of data derived from too few patients.
- A study will be undertaken of whether patients rebound after 6 months off treatment.
- A similar trial (N = 85) in the United States is the fourth arm with vaccine alone, but studying chronic HIV infection. Another arm with both interventions (IL-2 and IL-2 + vaccine) studied them simultaneously; results are forthcoming. The idea of giving vaccine after IL-2 was to boost resistance of IL-2.

Estimating the Impact of Transmitted Non-Nucleoside Reverse Transcriptase Inhibitor Resistance on Viral "Set-point"

Dr. Simon D.W. Frost, University of California, San Diego

Viral set-point is the relatively constant plasma viral load maintained during chronic infection. High viral set-points are associated with high infectiousness, more rapid progression to AIDS, and poor response to treatment. Drug resistance mutations incur a fitness cost in replication rate, so that drug resistant viruses can be transmitted although maybe at a lower rate than wild type virus. This study was conducted to determine whether transmitted drug-resistant HIV is associated with lower viral load. The concept of a constant viral load during chronic infection is a gross oversimplification of viral dynamics. The investigators used a random effects model to obtain individual-level set-point. Rather than estimate time since infection, they controlled for duration of follow-up and for fluctuations, which gave nonlinear dynamics. The model did well at predicting high viral load at the beginning and low at the end. There was no effect of replicative capacity on viral set-point. Efavirenz resistance is associated with higher viral load. They visualized the effect of transmitted NNRTI resistance on viral load. The model gives a good fit to viral load data; it's a useful model that can be used for more than comparisons, e.g., it can predict set-points on a dynamic model. NNRTI resistance is associated with elevated viral load, whereas higher replicative capacity is not. NRTI and PI resistance are associated with lower—not higher—viral load. This is in conflict with the conventional wisdom that drug resistance is associated with lower viral fitness, and they still have no idea what mechanism induces this effect.

Discussion

- NNRTI resistance increases whether the virus is wild type or not.
- The difference between donor set-point and recipient set-point may play a part.

- When K103 mutation was introduced to the virus and competition with wild type virus studied, small fitness benefit was found that would lose to wild type.
- Transmitted drug resistance is stronger than acquired drug resistance.

Comparisons of HIV RNA Levels after Stopping Early and Effective HAART to HIV RNA in Natural History

Dr. Loïc Desquilbet, Bicêtre Hospital Le Kremlin-Bicêtre, France

These investigators compared the effect of HAART initiated during primary HIV-1 infection on HIV RNA outcome during HAART interruption, and compared the HIV RNA level reached 1 year after stopping early and effective HAART with HIV RNA reached in natural HIV-1 history after the same duration of infection. Of their cohort of 58 patients, 22% were women; median age was 33 years. HIV RNA one year after HAART interruption was independent of the time between infection and initiation of HAART, and independent of the duration of sustained virological response to HAART; At 36 months since infection, there was a slight difference in HIV RNA between natural history and after stopping HAART. There was no strong evidence that HAART initiated during primary infection lowers virologic set-point after interruption. A controlled trial is needed to determine whether early HAART better than deferred.

Discussion

- Duration of virological response to treatment was 17 months with 2.5 months between initiation of HAART and response.
- In the two study populations, historical controls and patients from current data are similar on factors such as viral load and CD4 cell count at baseline.
- A proposed study in Zambia is similar: they will compare acutely infected patients through historical data and have open-label 3-month therapy; half will be male, half female.
- Most patients had been off treatment for 12 months, but some may have resumed sooner. The used model takes into account this discontinuity.
- A difference of 0.15 log corresponds to 1 year of increase in viral load. Confidence interval can be computed for each set-point, but there may be a margin of 0.3 or 0.4.

Is HAART for Acute/Early HIV Infection Associated with Improved Outcomes after Treatment Discontinuation?

Dr. Rick M. Hecht, University of California, San Francisco

Treatment in early infection may improve protection by developing anti-HIV immune responses. Because of recruitment problems in doing clinical trial, they analyzed historical data. Subjects had enrolled at AIEDRP sites within 6 months of HIV seroconversion; they had taken HAART for about 12 weeks (treatment period varied among participants) and stopped for 4 or more weeks. Viral load and CD4 count were compared. Results showed that acute treatment (first 2 weeks) improves viral load and CD4 outcomes for at least 72 weeks. Treatment initiated in early HIV had more transient benefit: the viral load benefit waned by 48 weeks; the CD4 benefit remained

but was diminished by 72 weeks. These data suggest a prolonged benefit of HAART when initiated in acute HIV infection, and a more transitory benefit when treatment is initiated later in early infection. The point at which benefit is lost of sustained viral load and CD4 count remains to be better defined, as does the duration of benefit in those treated in acute HIV.

Discussion

- Only 13 patients met the criteria. Therefore the investigators might be over-adjusting, although the unadjusted analysis is very similar. Both analyses give a similar message, but it would be useful to repeat the study with larger numbers over a longer period.
- Results differ from those from France, and it's breaking out the early treatment group that makes the difference: If they had been all lumped together you'd see almost no benefit.
- The data are not firm enough to make policy recommendations. Results suggest a benefit during early infection, but we need a randomized controlled trial for the early group. The data suggest, but not conclusively, a benefit if treatment is started early.
- A sustained biological benefit may reflect only people with good control because the others are back on therapy; we need to look at drop-outs more closely.
- When all studies cluster to a minor or no benefit, you have to weigh that small benefit against the high cost, toxicity, risk of complications, and earlier resistance. Toxicity comes from long-duration therapy, not limited duration. There's always the risk of resistance, but it's quite low at this time.

Prevalence of Transmitted Antiretroviral Resistance in Antiretroviral-naïve Patients with HIV-1 Primary Infection in 2003

Dr. Anita Shet, Aaron Diamond AIDS Research Center

Transmission frequency of drug resistant HIV-1 appears to have decreased since 2000. Previously published data from patients presenting at this center from 1995 to 2003 were used to study time trends. The epidemiological update shows that: 27.1% are resistant to any drug; 15.2% to NRTI; 18.2% to NNRTI; 7.6% to PI; and 10.5% are multi-drug resistant. Between 1993 and 2003 resistance increased from 10% to 18%, most dramatically for NNRTI and multi-drug resistance. Untreated transmitters continue, as indicated by the prevalence of primary drug resistance, to remain high. But NNRTI resistance mutations and multi-drug resistant virus show substantial increases.

Discussion

- No phylogenetic analysis was done here. They considered the utility of protease and reverse transcriptase to look for clusters; there were none in this group.
- Results were similar in Montreal: mean viral load decreased and NNRTI resistance increased because treated persons stopped taking their medication—this is of great concern. The answer is economic—we need free access to medication, which has resulted in a decrease in Europe.

- Acute infection was not separated from early infection.
- About a third of the patients were antibody negative and two-thirds were antibody positive, so the true incidence may be higher.
- The incidence of drug resistance in Australia appears to be decreasing.
- Most multi-drug resistant patients are resistance-tested so their therapy is tailored to them.

Summary and Future Directions

Moderator: Dr. Rick M. Hecht, University of California, San Francisco

Session 1: Disease Staging

Co-Chairs: Dr. John Kaldor, National Centre in HIV Epidemiology & Clinical Research, University of New South Wales
Dr. Brandee Pappalardo, Blood Systems Research Institute, San Francisco

There are many ways to identify and recruit people at early stages in different settings, and we should make use of them all. In the past, we have focused on people clinically or on those in seroconversion, but 2 different approaches are RNA testing on all patients, and making better use of Western blot results to get better staging.

Discussion

- Opportunities are still arising from rapid testing, particularly in resource-limited settings.
- Definition problems persist—e.g., "staging"—which seems context-dependent. Being able to accurately describe each stage is important. The NATO network has been working on this and may help. However, universal staging may be distant.

Session 2: Immunology I

Co-Chairs: Dr. Marcus Altfeld, Massachusetts General Hospital
Dr. Philip Goulder, Partners AIDS Research Center

HIV has an impact on innate immunity. We need to look at functional correlates in the initial control of viremia. In our knowledge of what's happening, there's a gap between infection and seroconversion. Regarding, immunodominance in early infection, it is important to understand what is targeted early and why and to target vaccines accordingly. Forces influencing HIV evolution include: immune responses (HLA frequency and ability to inhibit viral replication) versus viral adaptation (protein structure and influence of mutations on viral fitness) in between kinetics of CD8 escape and reversion, and evolution in the host and in the population. For CD4 cooperation and CD8 cooperation, the timing of treatment is crucial. We need to know why some investigators see a benefit to early treatment and some do not.

Session 3: Immunology II

Co-Chairs: *Dr. Jay Levy, University of California, San Francisco*
Dr. Cara Wilson, University of Colorado, Health Sciences Center

Immunologic pressure is a major selection factor, as is "genetic dose" at seroconversion, influence of HLA on diversity of transmitted strains, escape mutations, and monoclonal antibodies that can neutralize primary isolates. Primary infected subjects show neutralization, which appears to occur via chemokines. Superinfection is associated with changes in ART susceptibility; furthermore, plasma viral load increased and CD4 fell. Dual infection occurred among 19% of sex workers, which could be identified early; dual infection is associated with elevated set-point.

Questions for future research

What are the clinical consequences of HIV-1 transmission in HLA matched and unmatched subjects?
Can these monoclonal antibodies be used therapeutically?
Can similar monoclonal antibodies be induced?
Can monoclonal antibodies neutralize across clades?
Can we generate chemokines in a vaccine?
How should dual and superinfection be defined?
How can behavioral factors be modified?
How does the timing of dual infection affect clinical outcome?
Which characteristics of the co-infecting viral strain influence disease progression?

Discussion

- Innate immunity on HIV is important, but more important is the aging immune system and consequent complications, e.g., cancer, opportunistic infections.
- Which cellular response is more important and does treatment affect any of that? There may be an interplay among all the immune forms. We're just starting to learn about the various cellular types and responses.
- With limited patient populations and a limited number of cells, it's important to measure these things concurrently to make the best use of resources.
- Standardization of assays is an important issue.
- The CTL presentations focused on everything but environment, and environment is crucial.
- The number of superinfections is increasing; and patients appear to do worse after superinfection. Are we only catching the patients who are sicker and may not be able to fight the virus? Or are there general deficiencies in superinfection that make it difficult to fight?
- Progress has been made in elucidating how CTL fights virus, but not the negative consequences. People continue to generate immune responses that do not control viremia, and those responses may have negative effects.
- Reduced viral load will reduce viral diversity, but why do we see no difference between viral load and CD4? One concept is that during high levels of viral infection, CD4, once destroyed, cannot be restored. The difficulty is that there is great heterogeneity in acute infection and in patients. Therefore we need large

patient groups, and we need proper controlled trials. Even if something doesn't work, it would be economical and useful to know that. Early treatment prevents escape, but when viral load rebounds you see recapitulation of diversity.
- Can treatment result in a durable effect? When patients come off therapy they control viral load for a period; we need to make that durable. However, the vast majority of patients don't control. We need to be sure which are the immune responses that count.
- In children, in contrast to adults, you can regenerate immune response.

Session 4: Virology, Epidemiology, and Research in Resource-poor Settings

Co-Chairs: *Dr. Jean-Pierre Routy, McGill University*
Dr. Roberto Badaró, Hospital Universitãrio Professor Edgard Santos, Federal University of Bahia

There is more *env* diversity among homosexual than heterosexual men; 57% of heterosexuals were infected with multi-drug resistant strains. HIV serostatus in MSM does not influence high-risk sexual behavior; however, change in sexual behavior early leads to decreased transmission. Recruitment strategies have been both unfocused and focused. Large-scale screening of high-risk populations will dramatically increase detection of early HIV. Resource-limited settings impose many challenges, but patients there show high levels of compliance with clinical follow-up and adherence when ART is available.

Questions for future research

- How do you bring about changes in behavior?
- What is the impact of this change?
- How better to routinely implement screening for early HIV infection based on viral RNA or p24 antigen testing?
- How can we increase HIV awareness among leaders to apply more resources to treatment?

Discussion

- If people are more infectious during acute infection, should they be treated differently than chronically infected patients? Should we treat or not treat?
- In San Diego, they are doing a good job of identifying primary HIV infection, and at best they find only 5%. We need immensely better recruitment techniques.
- Transmission to partners could be impeded by treating the partner. There are also symptoms—e.g., meningitis, fatigue—that can be treated temporarily with medication. Behavior change takes time.
- Authorities in New York City show little interest in early intervention. Early symptoms can be subtle (50% in one study were asymptomatic). People may be most transmissible before the infection can be detected. The incidence of new infections is extraordinarily high in subpopulations of New York City, and efforts to frequently screen these populations could pay off. But you're still dealing with people who will

allow themselves to be tested. What proportion of people test around the time of their seroconversion? Both papers show that in an STD clinic they looked for antibody; this should be reversed.
- We need partnership with the Department of Health. Many of the people would not test because they are foreign-born and have no papers.

Session 5: Treatment and Resistance

Co-Chairs: *Dr. Susan Little, University of California, San Diego*
Dr. Rodney E. Phillips, University of Oxford

Treatment

Despite studies we lack answers to whether intervention with ART in the acute phase produces durable results. We need randomized clinical trials (and, The Wellcome Trust is going to fund such a study). Heterogeneity of infection in humans makes any study with few subjects difficult to interpret.

Drug resistance

We need continual studies to monitor the presence of transmitted drug resistance. We have progressed in standardization of definitions, but could use more work. More uniform definitions are helping interpret data among sites. It is still clear that there will be geographic differences. We need to continue to monitor in different risk groups using uniform definitions. What is the effect of ongoing treatment studies within the community in the context of ongoing transmission? The persistence of drug resistance may be an increasing problem regarding response to therapy over time—the effect of drug resistance variety on disease resistance; the effect of newly infected patients gives epidemic spread in a limited population; the effect of introduction of ART in a limited population must be monitored

Discussion

- NIH will fund a study of 150 patients for 9 months of treatment.
- How can we interpret the effects of drug resistance when we don't know the regimens used? Tracking what's transmitted from whom will be easier with standardized treatment.
- A Swiss study shows ethnic clustering of B subtypes; U.S. cohorts show more population mixing; and small cities show different things.

(Whereupon the meeting was adjourned at 3:00 p.m.)